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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Lisa B. Arthur
Group	:	1655
Applicants	:	Bernard F. Mach et al.
Application No.	:	08/484,786 Confirmation No. 4894
Filed	:	June 7, 1995
For	:	DNA SEQUENCES CODING FOR THE DR β-CHAIN LOCUS OF THE HUMAN LYMPHOCYTE ANTIGEN COMPLEX AND POLYPEPTIDES, DIAGNOSTIC TYPING PROCESSES AND PRODUCTS RELATED THERETO

Hon. Commissioner for Patents
P.O. Box 2327
Washington, D.C. 22202

DECLARATION OF JACK L. STROMINGER, M.D., D.Sc.
UNDER 37 C.F.R. § 1.132

I, Jack L. Strominger, M.D., D.Sc., declare and state as follows:

1. I am a Higgins Professor of Biochemistry in the Department of Molecular & Cellular Biology at Harvard University, Cambridge, Massachusetts, United States. I am also a Professor in the Department of Cancer Immunology and AIDS at the Dana-Farber Cancer Institute, Boston, Massachusetts, United States. I teach

the advanced course in molecular immunology at Harvard. I have spent over 33 years studying immunology, and 20 years studying molecular biology of the immune system. More particularly, I have spent over 31 years studying human lymphocyte antigens ("HLA"), and was among the first, if not the first, to apply molecular biology techniques to the field of immunology. Further details of my academic and scientific careers are included in my Curriculum Vitae, attached as Exhibit A hereto.

2. I am Chairman of the Scientific Advisory Board of Peptimmune, formerly a wholly-owned subsidiary of Genzyme Corporation, Cambridge, Massachusetts, United States, but now an independent entity. Additionally, I have served as a member of the Scientific Advisory Committees of several other scientific companies and organizations, including the Steering Committee of the Biomedical Sciences Scientific Working Group of the World Health Organization and the Board of Scientific Counselors, National Institute for Arthritis, Metabolic and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, United States.

3. I was awarded a M.D. degree from Yale Medical School, New Haven, Connecticut, United States in 1948. I have received honorary D.Sc. degrees from Washington University, St. Louis, Missouri, United States; Albany Medical College, Albany, New York, United States; Trinity College, Dublin, Ireland, and an honorary M.A. from the University of Oxford, Oxford, England.

4. During the period between 1955 and 1964, I held positions as Assistant Professor (from 1955 to 1958), Associate Professor (from 1958 to 1960) and Professor (from 1960 to 1968) at Washington University School of Medicine, St. Louis, Missouri, United States, and then was a Professor and Chairman of the Department of Pharmacology at the University of Wisconsin, Madison, Wisconsin (from 1964 to 1964). I have been affiliated with Harvard University since 1968 (i.e., for 33 years), holding a position as Professor of Biochemistry in the Department of Biochemistry & Molecular Biology (now called the Department of Molecular & Cellular Biology). During the period between 1974 and 1977, I served as Director of Basic Sciences at the Sidney Farber Cancer Institute (now Dana-Farber Cancer Institute), Boston, Massachusetts, United States, and presently hold a position at Dana-Farber Cancer Institute as Professor in the Department of Cancer Immunology and AIDS. In 1991, I was a visiting Newton-Abraham Research Professor at the University of Oxford, Oxford, England. I presently serve as member of the University-wide Committee on Immunology at Harvard University.

5. In 1995, I was awarded the Albert Lasker Basic Medical Research Award, and in 1999 I was presented the JAPAN PRIZE, in recognition of my scientific work in molecular recognition of the immune system, including genetic engineering technology and application of those disciplines to the production of vaccines. My work relating to the isolation and structure of

Class I and Class II major histocompatibility complex ("MHC") proteins, for which these awards were given, included the first cloning of several MHC genes and led to the knowledge of molecular recognition in the immune system, as well as vaccines and diagnostics based thereon, and is the subject of numerous patents, including 13 United States patents. A list of these United States patents can be found in Exhibit A attached hereto.

6. I have authored more than 930 publications and review articles in a wide range of leading international journals. A list of these publications can be found in Exhibit A attached hereto.

7. I am a founding member of the HLA Nomenclature Committee, which is comprised of pioneers in the field of HLA including: Dr. Bernard F. Mach, Dr. J.A. Hansen, Dr. B. Dupont and other distinguished researchers in the field. Today, in addition to naming newly-sequenced HLA alleles, the committee is responsible for the generation and maintenance of a HLA sequence database, which includes a compilation of HLA sequences reported in publications listed in the WHO Nomenclature Reports. The HLA sequence database currently contains 1496 allele sequences, and, as of today, 376 HLA-DR-β Class II alleles have been named. In addition to including the actual HLA sequences, the database contains detailed information concerning the material from which the sequences were derived and data on the validation of the sequences. In this database, HLA sequences are arranged with

alleles of each locus being aligned to a reference sequence, in order to depict any identity between residues.

8. I am given to understand that United States patent application no. 08/484,786 ("the '786 application") was filed on June 7, 1995 as a continuation application of United States patent application no. 07/902,999 (filed June 23, 1992, now United States patent 5,503,976), which is a divisional of United States patent application no. 06/518,393 (filed July 23, 1983, now United States patent 5,169,941). I am also given to understand that the '786 application claims an earliest priority date of July 30, 1982, based on Great Britain application no. 8222066 (filed on July 30, 1982) and Great Britain application no. 8230441 (filed on October 25, 1982).

9. I have read and considered the '786 application (Exhibit B, attached hereto). I have read and considered the November 16, 2001 Office Action issued in the '786 application ("Office Action")(Exhibit C, attached hereto). I have also read and considered claims 76-102 of the '786 application, which I am informed and believe to have been pending as of the date of the Office Action. A copy of those claims is attached hereto as Exhibit D. I have also read and considered the Declaration of Richard Lathe, D.Sc., including Exhibits A-M, filed in conjunction with the January 22, 2001 Amendment and Response. A copy of that Amendment and Response and the Lathe Declaration are attached hereto as Exhibits E and F, respectively.

10. I consider a person of skill in the art pertaining to the '786 application to be one skilled in the art of molecular immunology, and in particular, the field of HLA antigens, as well as the field of DNA hybridization, as of July 30, 1982 (a date not long after the beginning of the research involving HLA). Such a person would have a Ph.D. degree at that time and several years of relevant laboratory experience ("a person of skill in the art").

11. I make this declaration to address the following concerns raised in the November 16, 2001 Office Action. These are:

(1) Whether, given the identification and characterization in the '786 application of three specific polymorphic regions and one specific conserved region from three specific HLA-DR-β chain alleles, including DNA sequences encoding those regions, a person of skill in the art, as of July 30, 1982, would appreciate common features for identifying HLA-DR-β chain alleles, other than those three specific alleles, and DNA sequences capable of hybridizing to any one of those three specific polymorphic regions of such other HLA-DR-β chain alleles;

(2) Whether a person of skill in the art, as of July 30, 1982, would have known how to conduct hybridization experiments which would prevent non-specific hybridization of DNA sequences particularly at low stringency conditions, and

(3) Whether a person of skill in the art, as of July 30, 1982, would appreciate that the full-length HLA-DR-β-A, HLA-DR-β-B, and HLA-DR-β-C DNA sequences taught in the '786 application would be useful for HLA-DR typing.

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12. The '786 application identifies and characterizes three specific polymorphic regions (i.e., amino acids 8-14, 26-32 and 72-78) and one specific conserved region (i.e., amino acids 39-45) and DNA sequences encoding those regions from three specific HLA-DR-β chain alleles (i.e., HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C). Based on that teaching, it is my opinion that a person of skill in the art as of July 30, 1982, would appreciate common features for identifying HLA-DR-β chain alleles, other than HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles, and DNA sequences capable of hybridizing to a polymorphic region selected from amino acids 8-14, 26-32 or 72-78 of such other HLA-DR-β chain alleles.

13. Based on the teachings of the '786 application, including the identification and characterization of three specific polymorphic regions and one specific conserved region from three HLA-DR-β chain alleles and the state of the art relating to human lymphocyte antigens and DNA hybridization as it existed as of July 30, 1982, I believe that a person of skill in

the art would have understood the utility of and known how to exploit the three polymorphic regions and one conserved region shared by the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles to successfully carry out HLA-DR typing using DNA sequences capable of hybridizing to a polymorphic region selected from amino acids 8-14, 26-32 or 72-78 of HLA-DR-β chain alleles other than those three alleles.

14. As a scientific researcher in the field of human lymphocyte antigens in July 30, 1982, I believe that a person of skill in the art at that time would have appreciated the existence of several different HLA-DR-β chain alleles. My belief is based on the fact that, as of July 30, 1982, serological and mixed lymphocyte reaction testing had revealed a high degree of polymorphism among HLA proteins. See Exhibits G to J, attached hereto.

15. Given the knowledge that multiple HLA alleles most-likely existed, a person of skill in the art would have understood that the highly conserved DNA sequence and three polymorphic DNA sequences taught in the '786 application would also be useful for the identification and characterization of additional HLA-DR-β chain alleles. Such identification and characterization could be carried out by such a person using then-conventional experimentation.

16. As a researcher in the HLA field at July 30, 1982, it is my opinion that DNA sequences encoding the conserved region taught in the '786 application could have been used in conjunction with both the techniques described in the application and other then-conventional methods, to probe cDNA libraries to identify additional HLA-DR-β chain alleles. DNA sequences encoding the identified polymorphic regions, or the specific DNA sequences for those regions, as identified in the '786 application for the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles, all HLA-DR B1 alleles, could then be used to distinguish among different HLA-DR-β chain alleles. Thus, the polymorphic and conserved regions identified in the '786 application would have been extremely useful for hybridization-based HLA-DR typing.

17. The Office Action states that:

> "[b]ecause of the polymorphic nature of these genes, there may be many different DR-beta chain sequences of which three is not representative."

See Exhibit C, page 4, second full paragraph. I disagree with this statement. As a specific example, although my HLA research employed a different experimental strategy than that of the '786 application, it would have been possible for me to use the conserved and polymorphic DNA sequences of the '786 application, to probe cDNA libraries in an effort to identify HLA-DR-β chain alleles other than the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles. Because the '786 application taught three regions of

polymorphism (which are known today to be present in almost all of the HLA-DR-B1, HLA-DR-B3, HLA-DR-B4, and HLA-DR-B5 alleles identified and sequenced as of January 2002), a person of skill in the art at July 30, 1982 would have been able to use these three polymorphic regions in combination with then-routine DNA hybridization techniques to distinguish and categorize newly identified HLA-DR-β chain alleles, while using the conserved DNA sequence, encoding amino acids ("aa") 39-45 to distinguish non-HLA-DR-β chain sequences. That conserved sequence would bind specifically at the 39-45 aa region of HLA-DR-β chain alleles other than the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles described in the '786 application.

18. My opinion is supported by the sequence alignment of 363 HLA-DR-β chain alleles identified as of January 11, 2002, which revealed that there is 96% conservation among HLA-DR-β chain alleles between aa 39-45. In fact, of the 363 sequenced and aligned HLA-DR-B1, HLA-DR-B3, HLA-DR-B4, and HLA-DR-B5 alleles, all but 14 alleles have 100% homology at the region of aa 39-45, which was first identified in the '786 application as a conserved region. Additionally, of the 14 inexact matches, 6 of the alleles differ only by a single amino acid substitution, and the remaining 8 alleles are all HLA-DR-β4 alleles. See, Exhibit K, HLA-DRB Protein Sequence Alignments, downloaded from the website for the HLA Informatics Group - The Anthony Nolan Trust,

www.anthonynolan.com. Exhibit K has been annotated to indicate the conserved and polymorphic regions of HLA-DR-β chain alleles, identified as of January 11, 2002, for HLA-DR-B1, HLA-DR-B3, HLA-DR-B4, and HLA-DR-B5 alleles. Those alleles are listed in the left-hand column of Exhibit K, and their respective amino acid sequences are designated. As indicated in Exhibit K, the conserved aa 39-45 region (shown in pink), first taught in the '786 application as being conserved, is shared by all but 14 of the represented HLA-DR-β chain alleles. Furthermore, the regions of aa 8-14 (shown in blue), aa 26-32 (shown in green), and aa 72-78 (shown in yellow) are indeed polymorphic among a majority of the HLA-DR-β chain alleles represented. (Note: The sequence information, within the three polymorphic regions, is not provided for all of the HLA-DR-β chain alleles listed in Exhibit K. Thus, determination of an exact % of HLA-DR-β alleles that contain polymorphic 8-14, 26-32 and 72-78 regions is not possible. Although one of skill in the art as of July 30, 1982, would have believed that all HLA-DR-β alleles are polymorphic at one or more of these sites. Nonetheless, a visual inspection of the table, clearly shows that the regions of amino acids 8-14, 26-32 and 72-78 regions are in fact polymorphic.)

19. As explained by Dr. Lathe, one of skill in the art as of July 30, 1982 would have appreciated that the conserved DNA sequences described in the '786 application would be useful to

serve as a positive control in the DNA hybridization assays, in order to eliminate false positives (i.e., random binding to any non-HLA DNA sequences). In describing the use of the conserved DNA sequences of the '786 application to define other HLA-DR-β chain DNA sequences via DNA hybridization assays, Dr. Lathe states:

> "[s]ome would be positive controls: consisting of know HLA-DR-β sequences, such as those described in the '786 application. and deposited in connection therewith."

See Exhibit F, paragraph 22. I share Dr. Lathe's opinion that non-HLA sequences could easily be distinguished by using the conserved 39-45 region of the HLA-DR-β chain alleles taught in the '786 application.

21. In fact, use of DNA sequences encoding the polymorphic and conserved regions identified in the '786 application, or the specific DNA sequences for those regions identified in the '786 application for the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles, would have proven highly successful in the identification of HLA-DR-β chain alleles and HLA-DR typing. I base this statement on the above-discussed published sequences of the HLA-DR proteins, and the fact that all but 14 of the sequenced and aligned HLA-DR-B1, HLA-DR-B3, HLA-DR-B4, and HLA-DR-B5 alleles have 100% homology at amino acids 38-45, while all of the sequenced and aligned HLA-DR-B1, HLA-DR-B3, HLA-DR-B4, and HLA-DR-B5 alleles have polymorphic regions which correspond to at least

one of the 8-14, 26-32 and 72-78 of the HLA-DR-β chain locus. See Exhibit K.

22. For all these reasons described above, and in view of the data presented in Exhibit K, it is my opinion that the polymorphic regions and the conserved region of the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles (and corresponding DNA sequences) identified and characterized in the '786 application are predictive beyond those specific alleles and DNA sequences for carrying the claimed HLA typing methods and kits using DNA sequences capable of hybridizing to a polymorphic region selected from amino acids 8-14, 26-32 or 72-78 of HLA-DR-β chain alleles other than those three alleles. The '786 application first identified and characterized polymorphic and conserved regions which proved to hold a structural and functional similarity shared among various HLA-DR-β chain alleles. Such similarity, in turn, provided information sufficient to allow a person of skill in the art, as of July 30, 1982, to identify HLA-DR-β chain alleles, other than the HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C alleles exemplified in the '786 application, as well as DNA sequences capable of hybridizing to any one of those polymorphic regions of such other HLA-DR-β chain alleles.

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23. It is my opinion that a person of skill in the art as of July 30, 1982 would have appreciated that in order to achieve "specific" DNA hybridization -- in contrast to "non-specific" DNA hybridization -- the hybridization conditions (i.e., temperature, salt and/or concentration, pH) employed during the hybridization process are critical. In fact, the person of skill in the art as of July 30, 1982 would have known that the hybridization conditions must be optimized using control DNA prior to carrying out the hybridization assays using a test DNA sample.

24. I agree whole-heartedly with Dr. Lathe's explanation regarding the state of art of hybridization-based molecular biology as of July 30, 1982. I further agree with Dr. Lathe's opinion regarding the use of the term "hybridization" in the '786 application. See Exhibit F, paragraphs 8 and 9.

25. As discussed in Dr. Lathe's Declaration, the concepts of DNA hybridization were well understood and could be employed to generate hybrid DNA molecules as of July 30, 1982. See Exhibit F, paragraphs 8-23, and Exhibits A-M thereto.

26. I am in total agreement with Dr. Lathe's opinion that, as of July 30, 1982:

> "hybridization assays were of such routine nature in molecular biology labs . . . such assays would not involve undue experimentation."

See Exhibit F, paragraph 10. Additionally, I agree with the explanations provided by Dr. Lathe in paragraphs 11 to 23 of his declaration, including the Astell *et al.* and Szostak *et al.*

manuscripts. See also Exhibit F, paragraphs 10-11, and Exhibits G and H of Dr. Lathe's Declaration, respectively.

27. In order to identify additional HLA-DR-β chain alleles, one of skill in the art as of July 30, 1982 would have known to select hybridization conditions which would allow DNA hybrids to be formed between the control sequence and only highly homologous nucleotides (i.e., 100% shared identity), using then-routine techniques. More particularly, it is important to clarify that the conserved DNA sequence taught in the '786 application would bind specifically at, the control sequence, the 39-45 amino acid region of HLA-DR-β chain alleles other than those described in the '786 application.

28. As explained in Dr. Lathe's Declaration, the thermal stability of two hybridizing DNA sequences is dependent on a number of parameters, all of which were well known to the person of skill in the art as of July 30, 1982. See Exhibit F, paragraphs 11 and 12. Specifically, one of the most critical parameters is the concept of thermal stability, which involves the number of complementary base-pairs available to form a stable DNA duplex at any specific temperature. See Exhibit F, paragraph 18.

29. I agree whole-heartedly with Dr. Lathe's application of thermal stability to the concepts of DNA hybridization and DNA duplex formation to explain why the person of skill in the art as of July 30, 1982 would have know that the hybridization techniques of the '786 application would not be carried out under low

stringency hybridization conditions. As such, there is no scientific basis for the Examiner's assertion that the HLA-DR typing methods claimed in the '786 application would be in operable because: "any DNA molecule can hybridize to any other DNA molecule under low stringency hybridization conditions . . ."

30. Because the person of skill in the art as of July 30, 1982 would have understood the basic concepts of DNA hybridization, and how to employ hybridization techniques in conjunction with HLA-DR typing methods, it is my opinion that the claim recitation: "DNA sequences hybridize to specifically defined polymorphic regions of an HLA-DR-beta chain locus" would be fully understood by the person of skill in the art as of July 30, 1982. As such I believe the Examiner's assertion on this point to be without scientific merit.

31. Given the teachings of the '786 application and the well-understood concepts of DNA hybridization as of July 30, 1982, it is my opinion that a person of skill in the art would have known how to use the teachings of the '786 application, in conjunction with then-routine hybridization techniques, to avoid non-specific hybridization of DNA sequences in the HLA-DR typing methods and kits of the application.

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32. Based on the disclosure of the '786 application and the state of the art relating to human lymphocyte antigens, it is my opinion that a person of skill in the art, as of July 30, 1982, would appreciate that the full-length HLA-DR-β-A, DR-B, and DR-C sequences would be useful in methods for HLA-DR typing.

33. The disclosure of the '786 application states at pages 39 to 31, the exact steps that would be taken to carry out HLA DR typing methods, which employ the cDNA inserts HLA-DR-β-A, DR-B, and DR-C. In my opinion, there is no reason to expect that methods or kits characterized by those inserts would not be useful for HLA-DR typing.

34. Although the '786 application describes on pages 31 and 32 an "improved" HLA-DR typing method (i.e., employing synthetic probes), it is my opinion that this alternative HLA-DR typing method does not in anyway diminish the utility or importance of the HLA-DR typing carried out using the full-length cDNA inserts for HLA-DR-β-A, -B, or -C. For this reason, I believe that the Examiner's assertion that the full-length cDNA inserts of the HLA-DR-β sequences taught in the '786 application "would not be expected to be useful for typing", is scientifically unfounded.

CONCLUSION

35. Based on the above-cited reports, and my personal knowledge regarding the history of the identification of HLA genes, it is indisputable that the three polymorphic regions and the conserved region of the HLA-DR-β-A, HLA-DR-β-B, and HLA-DR-β-C alleles, first identified and characterized in the '786 application, served as very important tools for the identification of additional HLA-DR-β chain alleles and probes for hybridization-based HLA-DR typing.

36. I hereby further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing therefrom.

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Jack L. Strominger, M.D., D.Sc.

Signed this _____ day of _____, 2002

at _____, Massachusetts.